Genome-wide association study identifies five new schizophrenia loci

Ripke, Stephan; Sanders, Alan R.; Kendler, Kenneth S.; Levinson, Douglas F.; Sklar, Pamela; Holmans, Peter A.; Lin, Dan-Yu; Duan, Jubao; Ophoff, Roel A.; Andreassen, Ole A.

Published in:
Nature Genetics

DOI:
10.1038/ng.940

Publication date:
2011

nature genetics

Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure

Numerous genetic loci have been associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) in Europeans[1–3]. We now report genome-wide association studies of pulse pressure (PP) and mean arterial pressure (MAP). In discovery (N = 74,064) and follow-up studies (N = 48,607), we identified at genome-wide significance ($P = 2.7 \times 10^{-8}$ to $P = 2.3 \times 10^{-13}$) four new PP loci (at 4q12 near *CHIC2*, 7q22.3 near *PIK3CG*, 8q24.12 in *NOV* and 11q24.3 near *ADAMTS8*), two new MAP loci (3p21.31 in *MAP4* and 10q25.3 near *ADRB1*) and one locus associated with both of these traits (2q24.3 near *FIGN*) that has also recently been associated with SBP in east Asians. For three of the new PP loci, the estimated effect for SBP was opposite of that for DBP, in contrast to the majority of common SBP- and DBP-associated variants, which show concordant effects on both traits. These findings suggest new genetic pathways underlying blood pressure variation, some of which may differentially influence SBP and DBP.

High blood pressure is a major risk factor for coronary heart disease and stroke[4]. Large genome-wide association studies in Europeans have reported 29 new loci for SBP and DBP, in which alleles have effect sizes of up to 0.5–1.0 mm Hg[1–3]. Even small increments in blood pressure levels have important effects on cardiovascular morbidity and mortality at the population level[5]. We undertook a genome-wide association study of two further blood pressure phenotypes, PP (the difference between SBP and DBP), a measure of stiffness of the main arteries and MAP, a weighted average of SBP and DBP. Both PP and MAP are predictive of hypertension[6] and cardiovascular disease[7–9].

This study was undertaken by the International Consortium of Blood Pressure Genome-Wide Association Studies (ICBP-GWAS), which aims to further the understanding of the genetic architecture underlying blood pressure. A concurrent publication by this consortium[1] studied SBP and DBP with discovery GWAS among 69,395 people and a combined sample of ~200,000 Europeans. All but one study that was included in the discovery GWAS of SBP and DBP were included in the discovery GWAS stage of this study. In addition, we included here a further six studies added subsequent to the analyses of SBP and DBP[1], bringing our discovery GWAS sample size to 74,064.

We first conducted a genome-wide association meta-analysis of PP and MAP in these 74,064 individuals of European ancestry from 35 studies (**Supplementary Table 1a**). We imputed the genotypes using HapMap. To account for effects of anti-hypertensive treatments, we imputed underlying SBP and DBP by adding a constant to each[2,3]. We adjusted the associations for age, age^2, sex and body mass index. We combined the results across studies using an inverse-variance–weighted meta-analysis and, to correct for residual test statistic inflation, applied genomic control (GC) both to study-level association statistics and to the meta-analysis (genomic control inflation factor, λ_{GC} = 1.08. for PP and λ_{GC} = 1.12 for MAP)[10]. The quantile-quantile plots show an excess of extreme values largely accounted for by a modest number of genomic regions (**Supplementary Fig. 1a**,**b**). We performed independent follow-up analyses in 48,607 individuals of European ancestry (Online Methods and **Supplementary Note**).

SNPs in 12 regions showed genome-wide significant association ($P < 5 \times 10^{-8}$) with either PP or MAP in our discovery data (stage 1) (**Supplementary Fig. 1c**,**d**), including two previously unidentified regions for PP (7q22.3 near *PIK3CG*, $P = 1.2 \times 10^{-10}$ and 11q24.3 near *ADAMTS8*, $P = 8.5 \times 10^{-11}$; **Table 1**) and 10 regions previously associated with SBP and DBP (see **Supplementary Table 2a** for PP and **Supplementary Table 2b** for MAP)[1–3]. For follow-up in a series of independent cohorts, we selected 99 SNPs comprising those with $P < 1 \times 10^{-5}$ for either PP or MAP and SNPs reported in recent large genome-wide association studies of SBP and DBP[1–3] to evaluate their effects on PP and MAP (stage 2; Online Methods and **Supplementary Note**).

After the meta-analysis of the stage 1 and 2 data together (**Supplementary Table 2c**), the two new regions showing genome-wide association with PP after stage 1 (near *PIK3CG* and near *ADAMTS8*) remained genome-wide significant. In addition, we found genome-wide significant associations for SNPs at two further new loci for PP (at 4q12 near *CHIC2* and 8q24.12 in *NOV*), two new loci for MAP (3p21.31 in *MAP4* and 10q25.3 near *ADRB1*) and one locus for both traits (2q24.3 near *FIGN*) (**Table 1** and **Fig. 1**), a locus which has not previously shown an association with SBP or DBP in Europeans but which has recently been associated with SBP in east Asians (**Supplementary Note**)[11]. Forest plots of the stage 1 effect sizes and standard errors are shown in **Supplementary Figure 2**. The new signals for MAP were strongly associated with both SBP and DBP ($P = 7.7 \times 10^{-7}$ to $P = 1.8 \times 10^{-12}$), reflecting the high intercorrelations among these three blood pressure traits[12,13]. For the sentinel SNPs in three of the new PP loci, the estimated effects on SBP were in the opposite direction to the effects on DBP (**Table 1**, **Fig. 2** and **Supplementary Table 2d**,**e**). Our findings show that analyses of PP and MAP identify loci influencing blood pressure phenotypes that may not be detectable by studying SBP and DBP separately.

A full list of authors and affiliations appears at the end of the paper.

Received 10 February; accepted 4 August; published online 11 September 2011; doi:10.1038/ng.922

Table 1 Top genome-wide association results for pulse pressure and mean arterial pressure

Locus	Coded allele (frequency)	Stage 1			Stage 2			Stages 1+ 2			SBP stages 1+2		DBP stages 1+2	
		N_{eff}	β (s.e.m.)	*P*	N_{eff}	β (s.e.m.)	*P*	N_{eff}	β (s.e.m.)	*P*	β (s.e.m.)	*P*	β (s.e.m.)	*P*
Pulse pressure														
rs13002573 near *FIGN*, chr2:164,623,454	G (0.203)	73,043	–0.320 (0.07)	5.43×10^{-6}	43,955	–0.296 (0.089)	8.58×10^{-4}	116,998	–0.310 (0.055)	**1.76×10^{-8}**	–0.416 (0.081)	3.25×10^{-7}	–0.107 (0.052)	4.02×10^{-2}
rs871606 near *CHIC2*, chr4:54,494,002	T (0.85)	71,444	0.428 (0.096)	9.28×10^{-6}	44,082	0.431 (0.121)	3.75×10^{-4}	115,525	0.429 (0.075)	**1.32×10^{-8}**	0.403 (0.112)	3.04×10^{-4}	–0.010 (0.072)	8.85×10^{-1}
rs17477177 near *PIK3CG*, chr7:106,199,094	T (0.717)	72,997	–0.460 (0.071)	**1.19×10^{-10}**	39,999	–0.344 (0.094)	2.72×10^{-4}	112,996	–0.418 (0.057)	**2.27×10^{-13}**	–0.552 (0.084)	**5.67×10^{-11}**	–0.081 (0.055)	1.40×10^{-1}
rs2071518 *NOV* (3′ UTR), chr8:120,504,993	T (0.167)	73,252	0.304 (0.067)	5.72×10^{-6}	45,804	0.323 (0.086)	1.60×10^{-4}	119,056	0.312 (0.053)	**3.66×10^{-9}**	0.181 (0.078)	2.08×10^{-2}	–0.145 (0.050)	3.89×10^{-3}
rs11222084 near *ADAMTS8*, chr11:129,778,440	T (0.375)	67,704	0.415 (0.064)	**8.45×10^{-11}**	40,391	0.211 (0.081)	9.17×10^{-3}	108,095	0.337 (0.05)	**1.90×10^{-11}**	0.263 (0.074)	4.00×10^{-4}	–0.101 (0.048)	3.44×10^{-2}
Mean arterial pressure														
rs1446468 near *FIGN*, chr2:164,671,732	T (0.534)	69,264	–0.291 (0.061)	1.68×10^{-6}	39,650	–0.418 (0.082)	3.80×10^{-7}	108,914	–0.336 (0.049)	**6.46×10^{-12}**	–0.499 (0.071)	**1.82×10^{-12}**	–0.265 (0.046)	**6.88×10^{-9}**
rs319690 *MAP4* (intron), chr3:47,902,488	T (0.51)	59,137	0.306 (0.066)	3.88×10^{-6}	34,359	0.280 (0.09)	1.89×10^{-3}	93,496	0.297 (0.053)	**2.69×10^{-8}**	0.423 (0.077)	**4.74×10^{-8}**	0.282 (0.05)	**1.84×10^{-8}**
rs2782980 near *ADRB1*, chr10:115,771,517	T (0.198)	61,284	–0.345 (0.071)	1.14×10^{-6}	37,788	–0.326 (0.094)	5.55×10^{-4}	99,072	–0.338 (0.057)	**2.46×10^{-9}**	–0.406 (0.082)	7.66×10^{-7}	–0.283 (0.053)	9.60×10^{-8}

Pulse pressure (PP) and mean arterial pressure (MAP) association results from stages 1 and 2, and stages 1 and 2 combined, for all SNPs that showed genome-wide significant ($P < 5 \times 10^{-8}$) association with PP and/or MAP in the combined analysis and which had not previously been reported for systolic (SBP) or diastolic blood pressure (DBP). Also shown are the SBP and DBP combined stages 1 and 2 association results based on the same sample set as for PP and MAP (the full SBP and DBP results are listed in **Supplementary Table 2d,e**). Genome-wide significant associations ($P < 5 \times 10^{-8}$) are shown in bold. N_{eff}, *N* effective; UTR, untranslated region.

Identification of new genetic associations could help inform understanding about possible distinct mechanisms underlying relationships of PP with vascular risk[14,15].

Five additional loci for PP and 19 loci for MAP reaching genome-wide significance ($P < 5 \times 10^{-8}$ for stage 1 and 2 combined) were recently shown to be associated with SBP and/or DBP[1–3] (**Supplementary Table 2a**,**b**). We used sentinel SNPs from both the new and known regions showing genome-wide significant associations with PP or MAP in the combined stage 1 and 2 data to create weighted risk scores for PP (10 independent SNPs) and MAP (22 SNPs) (**Supplementary Table 2f**). We studied the associations of both risk scores with hypertension and blood pressure–related outcomes including coronary heart disease, heart failure, stroke, echocardiographic measures of left ventricular structure, pulse wave velocity, renal function and renal failure. Adjusting for multiple testing for the 12 traits evaluated ($P = 0.05/12 = 4.1 \times 10^{-3}$), the PP SNP risk score was associated with prevalent hypertension ($P = 7.9 \times 10^{-6}$), incident stroke ($P = 4.9 \times 10^{-4}$) and coronary heart disease ($P = 4.3 \times 10^{-4}$), and the MAP SNP risk score was associated with hypertension ($P = 5.1 \times 10^{-16}$), coronary heart disease ($P = 4.0 \times 10^{-20}$), stroke ($P = 0.0019$) and left ventricular wall thickness ($P = 2.1 \times 10^{-4}$) (**Supplementary Table 3a**), confirming the clinical relevance of these measures of blood pressure phenotype[8,9]. For a range of blood pressure–related outcomes (**Supplementary Note**), we compared *P* values for the PP risk score and a series of 1,000 permutations of SBP risk scores, each based on 10 of the 26 blood pressure SNPs associated with SBP but not with PP, and constraining the selection of SNPs to have similar sized effects for SBP as the 10 SNPs for PP. The PP risk score had a significantly ($P < 0.05$) greater association with risk of ischemic stroke than the SBP risk score (**Supplementary Note** and **Supplementary Table 3b**).

None of the genes in the identified newly associated regions is a strong candidate for blood pressure regulation, although several of them are implicated in mechanisms that may influence blood pressure. The most significant association with PP is within a putative mRNA clone (AF086203) spanning ~13.7 kb at 7q22.3, 94 kb upstream of *PIK3CG* (rs17477177, $P = 2.3 \times 10^{-13}$; **Table 1** and **Fig. 1a**). *PIK3CG* encodes the phosphoinositide-3-kinase, catalytic, γ polypeptide protein (PI3Kγ), which phosphorylates phosphoinositides and modulates extracellular signals. This region was earlier associated with mean platelet volume, platelet count and platelet aggregation[16–18], but the sentinel SNPs reported in those previous studies are independent of the SNP reported here, rs17477177 ($r^2 < 0.01$). Mice lacking the catalytic subunit of PI3Kγ have shown resistance to the SBP-lowering effects of β-adrenergic receptor agonists[19]; PI3Kγ activity is increased in the failing human heart and is associated with downregulation of β-adrenergic receptors in the plasma membrane[20]. The second locus for PP, located at 11q24.3, spans 35.5 kb, with the top-ranking SNP (rs11222084, $P = 1.9 \times 10^{-11}$; **Fig. 1b**) lying 1.6 kb downstream of *ADAMTS8*. This gene is highly expressed in macrophage-rich areas of human atherosclerotic plaques and may affect extracellular matrix remodeling[21]. The third locus for PP spans 28.5 kb at 8q24.12, with the sentinel SNP (rs2071518, $P = 3.7 \times 10^{-9}$; **Fig. 1c**) located in the 3′ untranslated region of *NOV*, encoding the nephroblastoma overexpressed (CCN3) protein, which is associated with angiogenesis, proliferation and inhibition of vascular smooth muscle cell growth and migration[22] and with reduced neointimal thickening in mice null for CCN3[23]. Mice with mutations in *Nov* that truncate the NOV protein show abnormal cardiac development[24]. Of the genes evaluated for expression in human aortic samples at the new PP loci, *NOV* showed by far the highest expression levels (**Supplementary Note** and **Supplementary Fig. 3**). The fourth locus for PP is 4q12, with the top-ranking SNP (rs871606, $P = 1.3 \times 10^{-8}$; **Fig. 1d**) located 76.7 kb downstream of *CHIC2*, encoding a cysteine-rich hydrophobic domain–containing protein that is associated with acute myeloid leukemia[25]. This SNP is located 296 kb upstream of *PDGFRA*, which encodes platelet-derived growth factor receptor α, a cell surface receptor for members of the platelet-derived growth factor family involved in kidney development. Variants in *PDGFRA* have

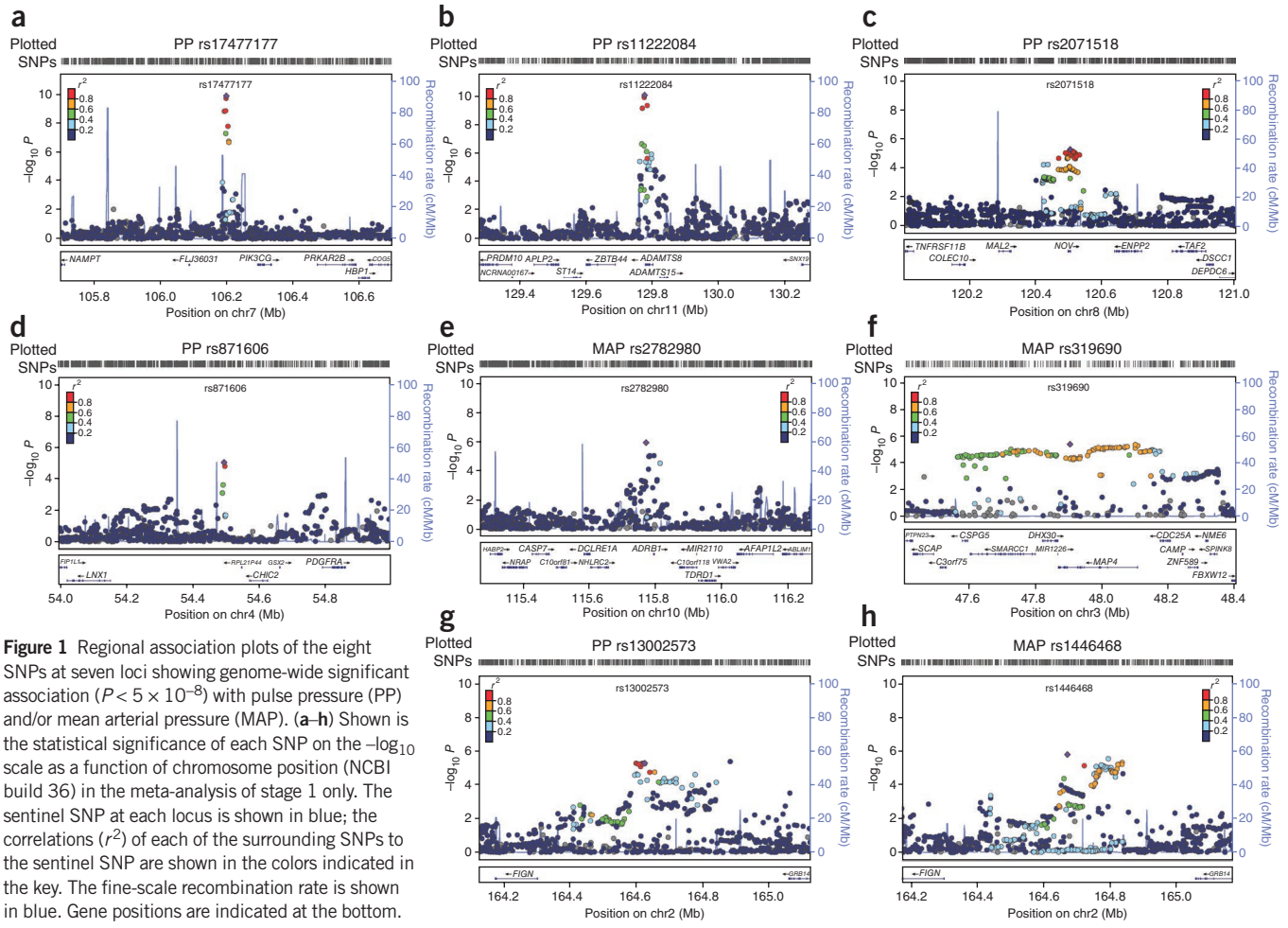

Figure 1 Regional association plots of the eight SNPs at seven loci showing genome-wide significant association ($P < 5 \times 10^{-8}$) with pulse pressure (PP) and/or mean arterial pressure (MAP). (**a**–**h**) Shown is the statistical significance of each SNP on the $-\log_{10}$ scale as a function of chromosome position (NCBI build 36) in the meta-analysis of stage 1 only. The sentinel SNP at each locus is shown in blue; the correlations (r^2) of each of the surrounding SNPs to the sentinel SNP are shown in the colors indicated in the key. The fine-scale recombination rate is shown in blue. Gene positions are indicated at the bottom.

been associated with red blood cell count and other haematological indices[26] but are independent ($r^2 < 0.3$) of rs871606.

For MAP, we identified two newly associated loci. The first locus for MAP is at 10q25.3, 22.3 kb upstream of *ADRB1* (rs2782980, $P = 2.5 \times 10^{-9}$; **Fig. 1e**). *ADRB1* encodes the β-1-adrenergic receptor, which mediates the effects of the stimulatory G protein and cAMP/protein kinase A pathway to increase heart rate and myocardial contraction. Polymorphisms in this gene have been associated with resting heart rate, response to beta blockers[27] and hypertension[28]. *Adrb1* knockout mice have no difference in heart rate or blood pressure compared with wild type but do have a significant reduction in the response of both phenotypes to catecholamines[29]. rs2782980 is associated with expression of an *ADRB1* transcript in brain tissue (**Supplementary Note** and **Supplementary Fig. 4a**). The second locus for MAP spans over 300 kb at 3p21.31, with the top-ranking SNP (rs319690, $P = 2.7 \times 10^{-8}$; **Fig. 1f**) lying within an intron of *MAP4*, encoding microtubule-associated protein 4. Coating of microtubules by MAP4 may inhibit β-adrenergic–receptor recycling and number, as seen in cardiac hypertrophy and failure[30]. *MAP4* was detectably expressed in human aortic samples (**Supplementary Note** and **Supplementary Fig. 3**).

The locus associated both with PP (rs13002573, $P = 1.8 \times 10^{-8}$; **Fig. 1g**) and MAP (rs1446468, $P = 6.5 \times 10^{-12}$; **Fig. 1h**) is in an intergenic region spanning ~280 kb at 2q24.3. Although the two signals are

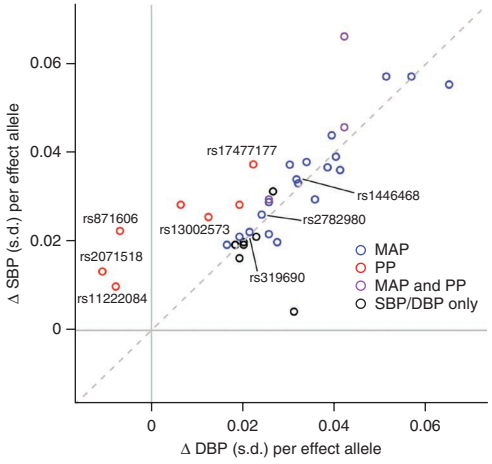

Figure 2 Systolic blood pressure (SBP) and diastolic blood pressure (DBP) effect sizes (β coefficients) for all blood pressure SNPs identified in the present study and a concurrent study[1] obtained from follow-up samples only. β coefficients are shown as standard deviation (s.d.) differences so that SBP and DBP are measured on comparable scales. Points are color coded according to whether they are genome-wide significant ($P < 5 \times 10^{-8}$) for pulse pressure (PP) (red), mean arterial pressure (MAP) (blue) or both MAP and PP (purple) in stages 1 and 2 of the present study, whereas those that are significant only for SBP and/or DBP from the concurrent study[1] are shown in black. The new SNPs found in the present study are labeled with their rs numbers. For illustration purposes, the effect allele for each SNP is defined such that the direction of the SBP effect is always positive.

~50 kb apart and are statistically independent ($r^2 = 0.075$), rs13002573 is highly correlated with rs16849225 ($r^2 = 1$ in the HapMap CEU population and $r^2 = 0.87$ in the HapMap JPT+CHB population), which has recently been reported as showing association with SBP in a GWAS of 19,608 subjects of east Asian origin with follow up in a further 30,765 individuals (combined $P = 3.5 \times 10^{-11}$) (ref. 11 and **Supplementary Note**). In our combined dataset of 116,998 Europeans, the association P value for rs13002573 with SBP was $P = 3.25 \times 10^{-7}$. The top PP SNP lies ~320 kb upstream of *FIGN* and ~430 kb downstream of *GRB14* (encoding growth factor receptor-bound protein 14). Relatively little is known regarding *FIGN* (encoding fidgetin).

We report six new loci associated with PP and MAP based on genome-wide discovery and follow-up in over ~120,000 individuals and a further locus (near *FIGN*) not previously reported in Europeans. Our results expand the knowledge of the genetic architecture of blood pressure and PP regulation and may give clues as to possible targets for blood pressure therapies.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

A number of the participating studies and authors are members of the CHARGE and Global BPgen consortia. Many funding mechanisms by the US National Institutes of Health and National Heart, Lung, and Blood Institute, European and private funding agencies contributed to this work, and a full list of acknowledgements is provided in the **Supplementary Note**.

AUTHOR CONTRIBUTIONS

ICBP-GWAS PP and MAP Working and Writing Sub-Group (alphabetical order): M.J.C., P.E. (co-chair), T.J., P.B.M., P.F.O., M.D.T. (co-chair), C.M.v.D. (co-chair), G.C.V., L.V.W. **ICBP-GWAS Steering Committee (alphabetical order):** G.R.A., M. Bochud, M. Boehnke, M.J.C. (co-chair), A.C., G.B.E., P.E., T.B.H., M.-R.J., A.D.J., T.J., M.G.L., L.L., D.L. (co-chair), P.B.M.(co-chair), C.N.-C. (co-chair), B.M.P., K.M.R., A.V.S., M.D.T., C.M.v.D., G.C.V. **Analysis:** L.V.W., G.C.V., P.F.O., T.J. **Expression analyses:** V.E., P.H., A.D.J., D.L., J.H.L., C.P.N., A. Plump, P.A.C.'t H., K.W.v.D. **Cohort contributions (alphabetical order): Study concept/design:** *AGES:* T.A., V.G., T.B.H., L.L., A.V.S. *AortaGen Consortium:* G.F.M. *ARIC:* E.B., A.C., S.K.G. *ASPS:* H. Schmidt, R.S. *BLSA:* L.F. *B58C-T1DGC:* D.P.S. *B58C-WTCCC:* D.P.S. *BHS:* L.J.P. *CARDIoGRAM Consortium:* N.J.S. *C4D Consortium:* R. Clarke, *CHS:* J.C.B., N.L.G., B.M.P., K.M.R., K.D.T. *CHARGE Consortium Heart Failure Working Group:* N.L.S. *CoLaus:* V.M., P. Vollenweider, G. Waeber *CROATIA-Korcula:* C.H. *CROATIA-Split:* M. Boban, I.R. *CROATIA-Vis:* A.F.W. *DeCode Genetics:* H.H., K.S., G.T., U.T. *DGI controls:* D.A., L.G., C.N.-C. *ENGAGE:* J.E., I.R.K. *EGCUT:* H.A., A.M. *EPIC:* K.-T.K. *ERF:* B.A.O. *Fenland:* N.J.W. *FUSION:* M. Boehnke, F.S.C., R.N.B., J.T. *INGI CARL:* A.P.d'A., P. Gasparini *INGI-FVG:* A.P.d'A., P. Gasparini *INCHIANTI:* S. Bandinelli., Y.M. *KORA S3:* C.G., M. Laan, E.O. *KORA F4:* T.M., H.-E.W. *LifeLines:* R.P.S., M.M.v.d.K. *LOLIPOP:* J.C.C., P.E., J.S.K. *LBC1921/LBC1936:* I.J.D., J.M.S. *MICROS:* A. Pfeufer *MESA:* G.L.B., X.G., W.P. *MIGen controls:* O.M., C.J.O., V.S., D. Siscovick *NESDA:* B.W.P., H. Snieder *NEURO-CHARGE Consortium:* M. Breteler, M. Fornage *NFBC1966:* M.-R.J., P.Z. *NSPHS:* U.B.G. *NTR:* D.I.B., E.J.C.d.G. *ORCADES:* H.C., J.F.W. *PROCARDIS controls:* M. Farrall, A. Hamsten, J.F.P., H.W. *PROSPER/PHASE:* B.B., J.W.J., D. Stott *RSI/RSII/RSIII:* A. Hofman, C.M.v.D., J.C.M.W. *SardiNIA:* G.R.A., M.U. *SHIP:* M.D., H.K.K., R.R., U.V., H.V. *SUVIMAX:* P. Galan, S. Hercberg, P.M. *TwinsUK:* T.D.S. *WGHS:* P.M.R. *YFS:* M.K., T.L., O.T.R., J.V. **Phenotype data acquisition and quality control:** *AGES:* T.A., V.G., T.B.H., L.L. *ARIC:* A.C., S.K.G., A.C.M., D.C.R. *ASPS:* M. Loitfelder, R.S. *BLSA:* S.S.N. *B58C-T1DGC:* D.P.S. *B58C-WTCCC:* D.P.S. *BHS:* J.P.B., J.H. *C4D Consortium:* R. Clarke, J.C.H. *CHS:* B.M.P. *CoLaus:* M. Bochud, V.M., P. Vollenweider *CROATIA-Korcula:* C.H., O.P. *CROATIA-Split:* M. Boban, I.R. *DGI controls:* L.G., C.N.-C. *EGCUT:* H.A., A.K., A.M., M.-L.T. *EPIC:* N.J.W. *Fenland:* N.J.W. *FHS:* S.-J.H., M.G.L., D.L., R.S.V., T.J.W. *FUSION:* J.T. *INGI CARL:* A.F., F.F., P. Gasparini, S.U. *INGI FVG:* A.F., F.F., P. Gasparini, S.U. *INGI-Val Borbera:* C. Masciullo, C.S., D.T. *INCHIANTI:* A.M.C. *KORA S3:* C.G. *KORA F4:* A.D. *LifeLines:* M.M.v.d.K. *LOLIPOP:* J.C.C., J.S.K., J.S. *LBC1921/LBC1936:* I.J.D., L.M.L., J.M.S. *MICROS:* M. Facheris, A. Pfeufer *MESA:* G.L.B., X.G., W.P. *MIGen controls:* G.L., O.M., C.J.O., V.S., D. Siscovick *NESDA:* X. Lu, I.M.N., B.W.P., H. Snieder *NEURO-CHARGE Consortium:* M. Breteler, S.D., A.L.D., M. Fornage *NFBC1966:* P.E., M.-R.J., J. Laitinen, A. Pouta, P.Z. *NSPHS:* U.B.G. *NTR:* D.I.B., E.J.C.d.G., G. Willemsen *ORCADES:* S.H.W., J.F.W. *PROCARDIS controls:* J.F.P. *PROSPER/PHASE:* D. Stott, S.T. *RSI/RSII/RSIII:* F.U.S.M.-R., E.J.G.S., C.M.v.D., G.C.V., J.C.M.W. *SardiNIA:* M.O., M.U. *SHIP:* M.D., R.R., H.V. *SUVIMAX:* P. Galan, M. Lathrop *TwinsUK:* T.D.S. *WGHS:* P.M.R. *YFS:* M.K., T.L., O.T.R., J.V. **Genotype data acquisition and quality control:** *AGES:* A.V.S. *ARIC:* A.C., G.B.E., S.K.G., A.C.M., D.C.R., G.S. *ASPS:* P. Gider, H. Schmidt, M.Z. *BLSA:* D. Hernandez *B58C-T1DGC:* S. Heath, W.L.M. *B58C-WTCCC:* W.L.M. *BHS:* J.P.B., R.J.W. *C4D Consortium:* J.C.H., H.O. *CHS:* J.C.B., N.L.G., K.D.T. *CoLaus:* V.M., P. Vollenweider *CROATIA-Korcula:* C.H., O.P. *CROATIA-Split:* I.R. *CROATIA-Vis:* V.V. *DGI controls:* D.A., B.F.V. *EGCUT:* T.E., T.H. *EPIC:* N.J.W. *Fenland:* R.J.F.L., J. Luan, N.J.W. *FHS:* S.-J.H., M.G.L. *FUSION:* F.S.C. *INGI CARL:* A.P.d'A. *INGI FVG:* A.P.d'A. *INGI Val Borbera:* C. Masciullo, C.S., D.T. *INCHIANTI:* A.S. *KORA S3:* C.G., M. Laan, E.O. *KORA F4:* T.M., H.-E.W. *LifeLines:* B.Z.A. *LOLIPOP:* J.C.C., J.S.K., J.S., W.Z. *LBC1921/LBC1936:* G.D., I.J.D. *MICROS:* I.P. *MESA:* G.L.B., Y.-D.I.C., X.G. *MIGen controls:* G.L., O.M., C.J.O., V.S., D.S. *NESDA:* J.F., X. Lu, I.M.N., B.W.P., H. Snieder *NFBC1966:* P.E., M.-R.J., J. Laitinen, P.Z. *NTR:* D.I.B., E.J.C.d.G., J.-J.H., G. Willemsen *ORCADES:* H.C., J.F.W. *PROCARDIS controls:* A.G., J.F.P. *PROSPER/PHASE:* S.T. *RSI/RSII/RSIII:* F.R., A.G.U. *SardiNIA:* G.R.A. *SHIP:* H.K.K., U.V., H.V. *SUVIMAX:* S. Heath, M. Lathrop *TwinsUK:* M.M., S.-Y.S., N.S., F.Z. *WGHS:* D.I.C., A.N.P. *YFS:* T.L., O.T.R. **Data analysis:** *AGES:* T.A., A.V.S. *ARIC:* A.C., G.B.E., A.C.M., V.P., D.C.R., G.S. *ASPS:* P. Gider, H. Schmidt, M.Z. *BLSA:* T.T. *B58C-T1DGC:* D.P.S. *B58C-WTCCC:* D. Hadley, D.P.S. *BHS:* A.W.M., L.J.P., R.J.W. *C4D Consortium:* J.C.H., H.O., CHS: J.C.B., N.L.G., K.M.R. *CoLaus:* J.S.B., S. Bergmann, M. Bochud, T.J. *CROATIA-Korcula:* C.H., O.P. *CROATIA-Split:* C.H. *CROATIA-Vis:* V.V. *DGI controls:* P.A., C.N.-C., B.F.V. *EchoGen Consortium:* J.F.F. *EGCUT:* T.E., T.H. *ENGAGE:* M.P. *EPIC:* I.B., R.J.F.L., N.J.W., J.H.Z. *ERF:* A.C.J.W.J., Y.A. *Fenland:* R.J.F.L., J. Luan *FHS:* S.-J.H., M.G.L. *FUSION:* A.U.J. *INGI CARL:* N.P. *INGI FVG:* N.P. *INGI Val Borbera:* T.C., G.P., C.S., D.T. *KORA S3:* S.E., S.S. *KORA F4:* B.K. *LifeLines:* B.Z.A. *LOLIPOP:* J.C.C., J.S.K., X. Li, J.S., W.Z. *LBC1921/LBC1936:* L.M.L. *MICROS:* F.D.G.M. *MESA:* Y-D.I.C., X.G., W.P. *MIGen controls:* G.L. *NESDA:* J.F., X. Lu *NEURO-CHARGE Consortium:* S.D., A.L.D., M. Fornage *NFBC1966:* P.F.O. *NSPHS:*W.I. *NTR:* J.-J.H. *ORCADES:* P.N., S.H.W., J.F.W. *PROCARDIS controls:* M. Farrall, A.G., J.F.P. *PROSPER/PHASE:* J.W.J., S.T. *RSI/RSII/RSIII:* N.A., S.K., C.M.v.D., G.C.V. *SardiNIA:* J.L.B.-G. *SHIP:* U.V. *SUVIMAX:* T.J., P.M. *TwinsUK:* N.S., F.Z. *WGHS:* D.I.C., L.M.R., YFS: T.L., O.T.R.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

Published online at http://www.nature.com/naturegenetics/.
Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Ehret, G. *et al.* Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* advance online publication, doi: 10.1038/nature10405 (11 September 2011).
2. Levy, D. *et al.* Genome-wide association study of blood pressure and hypertension. *Nat. Genet.* **41**, 677–687 (2009).
3. Newton-Cheh, C. *et al.* Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet.* **41**, 666–676 (2009).
4. Lawes, C.M. *et al.* Blood pressure and the global burden of disease 2000. Part II: estimates of attributable burden. *J. Hypertens.* **24**, 423–430 (2006).
5. Rose, G. Strategies of prevention: the individual and the population. in *Coronary Heart Disease Epidemiology: from Aetiology to Public Health* (ed. Marmot M, E.P.) 631–41 (Oxford University Press, Oxford, UK, 2005).
6. Domanski, M.J. *et al.* Independent prognostic information provided by sphygmomanometrically determined pulse pressure and mean arterial pressure in patients with left ventricular dysfunction. *J. Am. Coll. Cardiol.* **33**, 951–958 (1999).
7. Domanski, M. *et al.* Pulse pressure and cardiovascular disease-related mortality: follow-up study of the Multiple Risk Factor Intervention Trial (MRFIT). *J. Am. Med. Assoc.* **287**, 2677–2683 (2002).
8. Franklin, S.S. *et al.* Single versus combined blood pressure components and risk for cardiovascular disease: the Framingham Heart Study. *Circulation* **119**, 243–250 (2009).
9. Lewington, S., Clarke, R., Qizilbash, N., Peto, R. & Collins, R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* **360**, 1903–1913 (2002).
10. Devlin, B. & Roeder, K. Genomic control for association studies. *Biometrics* **55**, 997–1004 (1999).
11. Kato, N. *et al.* Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. *Nat. Genet.* **43**, 531–538 (2011).

12. Sesso, H.D. *et al.* Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. *Hypertension* **36**, 801–807 (2000).
13. Darne, B., Girerd, X., Safar, M., Cambien, F. & Guize, L. Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. *Hypertension* **13**, 392–400 (1989).
14. Blacher, J. & Safar, M.E. Large-artery stiffness, hypertension and cardiovascular risk in older patients. *Nat. Clin. Pract. Cardiovasc. Med.* **2**, 450–455 (2005).
15. Dart, A.M. & Kingwell, B.A. Pulse pressure–a review of mechanisms and clinical relevance. *J. Am. Coll. Cardiol.* **37**, 975–984 (2001).
16. Johnson, A.D. *et al.* Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. *Nat. Genet.* **42**, 608–613 (2010).
17. Soranzo, N. *et al.* A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts, and function. *Blood* **113**, 3831–3837 (2009).
18. Soranzo, N. *et al.* A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. *Nat. Genet.* **41**, 1182–1190 (2009).
19. Oudit, G.Y. *et al.* Phosphoinositide 3-kinase γ-deficient mice are protected from isoproterenol-induced heart failure. *Circulation* **108**, 2147–2152 (2003).
20. Perrino, C. *et al.* Dynamic regulation of phosphoinositide 3-kinase-γ activity and β-adrenergic receptor trafficking in end-stage human heart failure. *Circulation* **116**, 2571–2579 (2007).
21. Wågsäter, D. *et al.* ADAMTS-4 and -8 are inflammatory regulated enzymes expressed in macrophage-rich areas of human atherosclerotic plaques. *Atherosclerosis* **196**, 514–522 (2008).
22. Ellis, P.D., Chen, Q., Barker, P.J., Metcalfe, J.C. & Kemp, P.R. *Nov* gene encodes adhesion factor for vascular smooth muscle cells and is dynamically regulated in response to vascular injury. *Arterioscler. Thromb. Vasc. Biol.* **20**, 1912–1919 (2000).
23. Shimoyama, T. *et al.* CCN3 inhibits neointimal hyperplasia through modulation of smooth muscle cell growth and migration. *Arterioscler. Thromb. Vasc. Biol.* **30**, 675–682 (2010).
24. Heath, E. *et al.* Abnormal skeletal and cardiac development, cardiomyopathy, muscle atrophy and cataracts in mice with a targeted disruption of the *Nov* (*Ccn3*) gene. *BMC Dev. Biol.* **8**, 18 (2008).
25. Cools, J. *et al.* Fusion of a novel gene, *BTL*, to *ETV6* in acute myeloid leukemias with a t(4;12)(q11-q12;p13). *Blood* **94**, 1820–1824 (1999).
26. Kamatani, Y. *et al.* Genome-wide association study of hematological and biochemical traits in a Japanese population. *Nat. Genet.* **42**, 210–215 (2010).
27. Dorn, G.W. II. Adrenergic signaling polymorphisms and their impact on cardiovascular disease. *Physiol. Rev.* **90**, 1013–1062 (2010).
28. Kitsios, G.D. & Zintzaras, E. Synopsis and data synthesis of genetic association studies in hypertension for the adrenergic receptor family genes: the CUMAGAS-HYPERT database. *Am. J. Hypertens.* **23**, 305–313 (2010).
29. Rohrer, D.K., Chruscinski, A., Schauble, E.H., Bernstein, D. & Kobilka, B.K. Cardiovascular and metabolic alterations in mice lacking both β1- and β2-adrenergic receptors. *J. Biol. Chem.* **274**, 16701–16708 (1999).
30. Cheng, G., Qiao, F., Gallien, T.N., Kuppuswamy, D. & Cooper, G. IV. Inhibition of β-adrenergic receptor trafficking in adult cardiocytes by MAP4 decoration of microtubules. *Am. J. Physiol. Heart Circ. Physiol.* **288**, H1193–H1202 (2005).

Louise V Wain[1,2,179], Germaine C Verwoert[3,4,179], Paul F O'Reilly[5,179], Gang Shi[6,7,179], Toby Johnson[8,179], Andrew D Johnson[9,10], Murielle Bochud[11,12], Kenneth M Rice[13], Peter Henneman[14], Albert V Smith[15,16], Georg B Ehret[17–19], Najaf Amin[20], Martin G Larson[9,21], Vincent Mooser[22], David Hadley[23,24], Marcus Dörr[25], Joshua C Bis[26], Thor Aspelund[15,16], Tõnu Esko[27–29], A Cecile J W Janssens[20], Jing Hua Zhao[30], Simon Heath[31], Maris Laan[29], Jingyuan Fu[32,33], Giorgio Pistis[34], Jian'an Luan[30], Pankaj Arora[35], Gavin Lucas[36], Nicola Pirastu[37], Irene Pichler[38], Anne U Jackson[39], Rebecca J Webster[40], Feng Zhang[41], John F Peden[42,43], Helena Schmidt[44], Toshiko Tanaka[45], Harry Campbell[46], Wilmar Igl[47], Yuri Milaneschi[45], Jouke-Jan Hottenga[48], Veronique Vitart[49], Daniel I Chasman[50,51], Stella Trompet[52,53], Jennifer L Bragg-Gresham[39], Behrooz Z Alizadeh[32], John C Chambers[5,54], Xiuqing Guo[55], Terho Lehtimäki[56], Brigitte Kühnel[57], Lorna M Lopez[58,59], Ozren Polašek[60], Mladen Boban[61], Christopher P Nelson[62], Alanna C Morrison[63], Vasyl Pihur[17], Santhi K Ganesh[64], Albert Hofman[20], Suman Kundu[20], Francesco U S Mattace-Raso[3,20], Fernando Rivadeneira[3,4], Eric J G Sijbrands[3,20], Andre G Uitterlinden[3,4], Shih-Jen Hwang[9,10,65], Ramachandran S Vasan[9,66], Thomas J Wang[9,67], Sven Bergmann[68,69], Peter Vollenweider[70], Gérard Waeber[70], Jaana Laitinen[71], Anneli Pouta[72,73], Paavo Zitting[74], Wendy L McArdle[75], Heyo K Kroemer[76], Uwe Völker[77], Henry Völzke[78], Nicole L Glazer[79], Kent D Taylor[55], Tamara B Harris[80], Helene Alavere[27], Toomas Haller[27], Aime Keis[27], Mari-Liis Tammesoo[27], Yurii Aulchenko[20], Inês Barroso[81,82], Kay-Tee Khaw[83], Pilar Galan[84–86], Serge Hercberg[84–86], Mark Lathrop[31], Susana Eyheramendy[87], Elin Org[29], Siim Sõber[29], Xiaowen Lu[32], Ilja M Nolte[32], Brenda W Penninx[88–90], Tanguy Corre[34], Corrado Masciullo[34], Cinzia Sala[34], Leif Groop[91], Benjamin F Voight[92], Olle Melander[93], Christopher J O'Donnell[94], Veikko Salomaa[95], Adamo Pio d'Adamo[37], Antonella Fabretto[96], Flavio Faletra[96], Sheila Ulivi[96], Fabiola Del Greco M[38], Maurizio Facheris[38], Francis S Collins[97], Richard N Bergman[98], John P Beilby[99–101], Joseph Hung[102,101], A William Musk[101,103,104], Massimo Mangino[41], So-Youn Shin[41,81], Nicole Soranzo[41,81], Hugh Watkins[42,43], Anuj Goel[42,43], Anders Hamsten[105], Pierre Gider[44], Marisa Loitfelder[106], Marion Zeginigg[44], Dena Hernandez[107], Samer S Najjar[108,109], Pau Navarro[49], Sarah H Wild[46], Anna Maria Corsi[110], Andrew Singleton[107], Eco J C de Geus[111], Gonneke Willemsen[111], Alex N Parker[112], Lynda M Rose[50], Brendan Buckley[113], David Stott[114], Marco Orru[115], Manuela Uda[115], LifeLines Cohort Study, Melanie M van der Klauw[116], Weihua Zhang[5,54], Xinzhong Li[5], James Scott[117], Yii-Der Ida Chen[55], Gregory L Burke[118], Mika Kähönen[119], Jorma Viikari[120], Angela Döring[121,122], Thomas Meitinger[123,124], Gail Davies[59], John M Starr[58,125], Valur Emilsson[15], Andrew Plump[126], Jan H Lindeman[127], Peter A C 't Hoen[14,128], Inke R König[129], EchoGen consortium[178], Janine F Felix[4,20,130], Robert Clarke[131], Jemma C Hopewell[131], Halit Ongen[42], Monique Breteler[20], Stéphanie Debette[132], Anita L DeStefano[133], Myriam Fornage[134], AortaGen Consortium[178], Gary F Mitchell[135], CHARGE Consortium Heart Failure Working Group[178], Nicholas L Smith[136–138], KidneyGen consortium[178], Hilma Holm[139], Kari Stefansson[139,140], Gudmar Thorleifsson[139], Unnur Thorsteinsdottir[139,140], CKDGen consortium[178], Cardiogenics consortium[178], CardioGram[178],

Nilesh J Samani[62,141], Michael Preuss[142,129], Igor Rudan[46,143], Caroline Hayward[49], Ian J Deary[58,59], H-Erich Wichmann[121,144], Olli T Raitakari[145], Walter Palmas[146], Jaspal S Kooner[54,117], Ronald P Stolk[147], J Wouter Jukema[52,148,149], Alan F Wright[49], Dorret I Boomsma[111], Stefania Bandinelli[150], Ulf B Gyllensten[47], James F Wilson[46], Luigi Ferrucci[45], Reinhold Schmidt[106], Martin Farrall[42,43], Tim D Spector[41], Lyle J Palmer[40,101,151,152], Jaakko Tuomilehto[153–155], Arne Pfeufer[38,156,157], Paolo Gasparini[37,96], David Siscovick[26,136,158], David Altshuler[92,159–161], Ruth J F Loos[30], Daniela Toniolo[34,162], Harold Snieder[32], Christian Gieger[57], Pierre Meneton[163], Nicholas J Wareham[30], Ben A Oostra[164], Andres Metspalu[27–29], Lenore Launer[165], Rainer Rettig[166], David P Strachan[23], Jacques S Beckmann[68,167], Jacqueline C M Witteman[4,20], Jeanette Erdmann[142], Ko Willems van Dijk[14,168], Eric Boerwinkle[169], Michael Boehnke[39], Paul M Ridker[50,51,170], Marjo-Riitta Jarvelin[5,171,172], Aravinda Chakravarti[17], Goncalo R Abecasis[39], Vilmundur Gudnason[15,16], Christopher Newton-Cheh[35,92], Daniel Levy[9,10,65], Patricia B Munroe[8,179], Bruce M Psaty[26,136,173,138,179], Mark J Caulfield[8,179], Dabeeru C Rao[6,7,174,175,179], Martin D Tobin[1,2,179], Paul Elliott[5,176,179] & Cornelia M van Duijn[4,20,177,179]

[1]Department of Health Sciences, University of Leicester, Leicester, UK. [2]Department of Genetics, University of Leicester, Leicester, UK. [3]Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands. [4]Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), The Netherlands. [5]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK. [6]Division of Biostatistics, Washington University in St. Louis, School of Medicine, Saint Louis, Missouri, USA. [7]Department of Genetics, Washington University in St. Louis, School of Medicine, Saint Louis, Missouri, USA. [8]Clinical Pharmacology and The Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK. [9]Framingham Heart Study, Framingham, Massachusetts, USA. [10]National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA. [11]Institute of Social and Preventive Medicine (IUMSP), Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. [12]University of Lausanne, Lausanne, Switzerland. [13]Department of Biostatistics, University of Washington, Seattle, Washington, USA. [14]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands. [15]Icelandic Heart Association, Kopavogur, Iceland. [16]University of Iceland, Reykajvik, Iceland. [17]Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [18]Institute of Social and Preventive Medicine, Lausanne University Hospital, Lausanne, Switzerland. [19]Cardiology, Department of Specialties of Internal Medicine, Geneva University Hospital, Geneva, Switzerland. [20]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. [21]Department of Mathematics, Boston University, Boston, Massachusetts, USA. [22]Genetics Division R&D, GlaxoSmithKline, King of Prussia, Pennsylvania, USA. [23]Division of Community Health Sciences, St George's, University of London, London, UK. [24]Pediatric Epidemiology Center, University of South Florida, Tampa, Florida, USA. [25]Department of Internal Medicine B, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. [26]Cardiovascular Health Research Unit, Division of Internal Medicine, Department of Medicine, University of Washington, Seattle, Washington, USA. [27]Estonian Genome Center, University of Tartu, Tartu, Estonia. [28]Estonian Biocenter, Tartu, Estonia. [29]Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia. [30]Medical Research Council (MRC) Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK. [31]Centre National de Génotypage, Commissariat à L'Energie Atomique, Institut de Génomique, Evry, France. [32]Unit of Genetic Epidemiology and Bioinformatics, Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [33]Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [34]Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milano, Italy. [35]Center for Human Genetic Research, Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts, USA. [36]Cardiovascular Epidemiology and Genetics, Institut Municipal d'Investigacio Medica, Barcelona Biomedical Research Park, Barcelona, Spain. [37]Institute for Maternal and Child Health–Istituto Di Ricovero e Cura a Carattere Scientifico (IRCCS) 'Burlo Garofolo'—Trieste, University of Trieste, Trieste, Italy. [38]Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy. Affiliated Institute of the University of Lübeck, Lübeck, Germany. [39]Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA. [40]Centre for Genetic Epidemiology and Biostatistics, University of Western Australia, Crawley, Western Australia, Australia. [41]Department of Twin Research and Genetic Epidemiology, King's College London, London, UK. [42]Department of Cardiovascular Medicine, The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK. [43]Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford, UK. [44]Institute of Molecular Biology and Biochemistry, Medical University Graz, Graz, Austria. [45]Clinical Research Branch, National Institute on Aging, Baltimore, Maryland, USA. [46]Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK. [47]Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden. [48]Neuroscience Campus Amsterdam (NCA), Department of Biological Psychology, VU University, Amsterdam, The Netherlands. [49]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK. [50]Division of Preventive Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA. [51]Harvard Medical School, Boston, Massachusetts, USA. [52]Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands. [53]Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands. [54]Ealing Hospital National Health Service (NHS) Trust, Middlesex, UK. [55]Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA. [56]Department of Clinical Chemistry, University of Tampere and Tampere University Hospital, Tampere, Finland. [57]Institute of Genetic Epidemiology, Helmholtz Zentrum München-German Research Center for Environmental Health, Neuherberg, Germany. [58]Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, Edinburgh, UK. [59]Department of Psychology, The University of Edinburgh, Edinburgh, UK. [60]Department of Public Health, Medical School, University of Split, Split, Croatia. [61]Department of Pharmacology, Medical School, University of Split, Split, Croatia. [62]Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK. [63]Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas at Houston Health Science Center, Houston, Texas, USA. [64]Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan Medical Center, Ann Arbor, Michigan, USA. [65]Center for Population Studies, National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA. [66]Division of Epidemiology and Prevention, Boston University School of Medicine, Boston, Massachusetts, USA. [67]Division of Cardiology, Massachusetts General Hospital, Boston, Massachusetts, USA. [68]Département de Génétique Médicale, Université de Lausanne, Lausanne, Switzerland. [69]Swiss Institute of Bioinformatics, Lausanne, Switzerland. [70]Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. [71]Finnish Institute of Occupational Health, Oulu, Finland. [72]National Institute of Health and Welfare, Department of Children, Young People and Families, Oulu, Finland. [73]Institute of Clinical Medicine, Obstetrics and Gynaecology, University of Oulu, Oulu, Finland. [74]Department of Physiatrics, Lapland Central Hospital, Rovaniemi, Finland. [75]Avon Longitudinal Study of Parents and Children (ALSPAC) Laboratory, Department of Social Medicine, University of Bristol, Bristol, UK. [76]Institute of Pharmacology, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. [77]Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. [78]Institute for Community Medicine, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. [79]Section of Preventive Medicine and Epidemiology, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA. [80]National Institute of Aging's Laboratory for Epidemiology, Demography and Biometry, Bethesda, Maryland, USA. [81]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK. [82]University of Cambridge Metabolic Research Labs, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK. [83]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge, UK. [84]U557 Institut National de la Santé et de la Recherche Médicale, Paris, France. [85]U1125 Institut National de la Recherche Agronomique, Paris, France. [86]Université Paris 13, Bobigny, France. [87]Department of Statistics, Pontificia Universidad Catolica de Chile, Santiago, Chile. [88]Department of Psychiatry/EMGO Institute/Neuroscience Campus, VU University Medical Centre, Amsterdam, The Netherlands. [89]Department of Psychiatry, Leiden University Medical Centre, Leiden, The Netherlands. [90]Department of Psychiatry, University Medical Center Groningen, University of Groningen, Groningen,

The Netherlands. [91]Department of Clinical Sciences, Diabetes and Endocrinology Research Unit, Lund University, University Hospital Malmö, Malmö, Sweden. [92]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. [93]Department of Clinical Sciences, Hypertension and Cardiovascular Diseases, University Hospital Malmö, Lund University, Malmö, Sweden. [94]National Heart, Lung, and Blood Institute and its Framingham Heart Study, Framingham, Massachusetts, USA. [95]Department of Chronic Disease Prevention, THL-National Institute for Health and Welfare, Helsinki, Finland. [96]Institute for Maternal and Child Health-Istituto Di Ricovero e Cura a Carattere Scientifico (IRCCS) 'Burlo Garofolo'—Trieste, Italy. [97]National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA. [98]Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California, USA. [99]Pathology and Laboratory Medicine, University of Western Australia, Crawley, Western Australia, Australia. [100]Molecular Genetics, PathWest Laboratory Medicine, Nedlands, Western Australia, Australia. [101]Busselton Population Medical Research Foundation, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia. [102]School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia, Australia. [103]School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia, Australia. [104]Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia. [105]Atherosclerosis Research Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden. [106]Department of Neurology Section of Special Neurology, Medical University Graz, Graz, Austria. [107]National Institute of Aging's Laboratory of Neurogenetics, Bethesda, Maryland, USA. [108]Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, NIH, Baltimore, Maryland, USA. [109]Cardiovascular Research Institute, MedStar Health Research Institute, Washington Hospital Center, Washington DC, USA. [110]Tuscany Regional Health Agency, Florence, Italy. [111]EMGO+ institute, Department of Biological Psychology, VU University, Amsterdam, The Netherlands. [112]Amgen, Cambridge, Massachusetts, USA. [113]Department of Pharmacology and Therapeutics, University College Cork, Cork, Ireland. [114]Institute of Cardiovascular and Medical Sciences, School of Medicine, University of Glasgow, Glasgow, UK. [115]Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Monserrato, Italy. [116]Department of Endocrinology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [117]National Heart and Lung Institute, Imperial College London, Hammersmith Hospital, London, UK. [118]Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA. [119]Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, Finland. [120]Department of Medicine, University of Turku and Turku University Hospital, Turku, Finland. [121]Institute of Epidemiology I, Helmholtz Zentrum München-German Research Center for Environmental Health, Neuherberg, Germany. [122]Institute of Epidemiology II, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany. [123]Institute of Human Genetics, Helmholtz Zentrum München–German Research Centre for Environmental Health, Neuherberg, Germany. [124]Institute of Human Genetics, Technische Universität München, Munich, Germany. [125]Geriatric Medicine Unit, The University of Edinburgh, Royal Victoria, Edinburgh, UK. [126]Cardiovascular Disease Franchise, Merck Research Laboratory, Rahway, New Jersey, USA. [127]Department of Vascular Surgery, Leiden University Medical Center, Leiden, The Netherlands. [128]Leiden Genome Technology Center, Leiden University Medical Center, Leiden, The Netherlands. [129]Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Lübeck, Germany. [130]German Cancer Research Center, Division of Clinical Epidemiology and Aging Research, Heidelberg, Germany. [131]Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, UK. [132]Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, USA. [133]Boston University School of Public Health, Boston, Massachusetts, USA. [134]Brown Foundation Institute of Molecular Medicine and Human Genetics Center, University of Texas Health Science Center at Houston, Houston, Texas, USA. [135]Cardiovascular Engineering, Inc., Norwood, Massachusetts, USA. [136]Department of Epidemiology, University of Washington, Seattle, Washington, USA. [137]Seattle Epidemiologic Research and Information, Center of the Department of Veterans Affairs Office of Research and Development, Seattle, Washington, USA. [138]Group Health Research Institute, Group Health Cooperative, Seattle, Washington, USA. [139]deCODE genetics Inc. Reykjavik, Iceland. [140]Faculty of Medicine, University of Iceland, Reykjavik, Iceland. [141]Leicester National Institute of Health Research (NIHR) Biomedical Research Unit in Cardiovascular Disease, Glenfield Hospital, Leicester, UK. [142]Medizinische Klinik II, Universität zu Lübeck, Lübeck, Germany. [143]Croatian Centre for Global Health, University of Split Medical School, Split, Croatia. [144]Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität and Klinikum Grosshadern, Munich, Germany. [145]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and the Department of Clinical Physiology, Turku University Hospital, Turku, Finland. [146]Columbia University, New York, New York, USA. [147]Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [148]Durrer Center for Cardiogenetic Research, Amsterdam, The Netherlands. [149]Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands. [150]Geriatric Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy. [151]Ontario Institute for Cancer Research, Toronto, Ontario, Canada. [152]Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada. [153]National Institute for Health and Welfare, Diabetes Prevention Unit, Helsinki, Finland. [154]Hjelt Institute, Department of Public Health, University of Helsinki, Helsinki, Finland. [155]South Ostrobothnia Central Hospital, Seinajoki, Finland. [156]Institute of Human Genetics, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany. [157]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany. [158]Department of Medicine, University of Washington, Seattle, Washington, USA. [159]Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA. [160]Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA. [161]Diabetes Unit, Massachusetts General Hospital, Boston, Massachusetts, USA. [162]Institute of Molecular Genetics–Consiglio Nazionale delle Ricerche (CNR), Pavia, Italy. [163]U872 Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris, France. [164]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands. [165]National Institute of Aging's Laboratory for Epidemiology, Demography and Biometry, Bethesda, Maryland, USA. [166]Institute of Physiology, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. [167]Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. [168]Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands. [169]Human Genetics Center, Houston, Texas, USA. [170]Division of Cardiology, Brigham and Women's Hospital, Boston, Massachusetts, USA. [171]Institute of Health Sciences, University of Oulu, Oulu, Finland. [172]Biocenter, University of Oulu, Oulu, Finland. [173]Department of Health Services, University of Washington, Seattle, Washington, USA. [174]Department of Psychiatry, Washington University in St. Louis, School of Medicine, Saint Louis, Missouri, USA. [175]Department of Mathematics, Washington University in St. Louis, School of Medicine, Saint Louis, Missouri, USA. [176]MRC-Health Protection Agency (HPA) Centre for Environment and Health, Imperial College London, London, UK. [177]Centre of Medical Systems Biology, Erasmus University Medical Center, Rotterdam, The Netherlands. [178]A full list of members is provided in the **Supplementary Note**. [179]These authors contributed equally to this work. Correspondence should be addressed to P.E. (p.elliott@imperial.ac.uk), M.D.T. (mt47@leicester.ac.uk) or C.M.v.D (c.vanduijn@erasmusmc.nl).

ONLINE METHODS

Phenotypes. PP was defined as systolic minus diastolic pressure, and MAP was defined as 2/3 diastolic plus 1/3 systolic pressure. A two-staged analysis was used to discover genes associated with PP and MAP.

Stage 1 samples and analyses. Stage 1 was a meta-analysis of directly genotyped and imputed SNPs from population-based or control samples from case-control studies in the International Consortium of Blood Pressure Genome-wide Association Studies (ICBP-GWAS). The characteristics of the 35 studies, including demographics, genotyping arrays, quality control filters and statistical analysis methods used, are listed in **Supplementary Table 1a**,**b**. Imputation of the allele dosage of ungenotyped SNPs in HapMap CEU v21a or v22 was carried out by each of the studies using MACH[31], IMPUTE[32] or BIMBAM[33] with parameters and pre-imputation filters as specified in **Supplementary Table 1b**. SNPs were excluded from analysis if the study-specific imputation quality (r2.hat in MACH or .info in IMPUTE) was <0.3. In total, up to 2,652,054 genotyped or imputed autosomal SNPs were analyzed. Full details of the models, methods and corrections for antihypertensive treatment are provided in the **Supplementary Note**. All analyses assumed an additive genetic model and were adjusted for sex, age, age^2, body mass index and ancestry principal components. In related individuals, regression methods that account for relatedness were applied. All study-specific effect estimates and coded alleles were oriented to the forward strand of the HapMap release 22, with the alphabetically higher allele as the coded allele. To capture loss of power caused by imperfect imputation, we estimated '*N* effective' as the sum of the study-specific products of the imputation quality metric and the sample size. No filtering on minor allele frequency was done. Genomic control was carried out on study-level data, and inverse-variance weighting was used for the meta-analysis of stage 1. The meta-analysis results were subject to genomic control. Genomic control inflation factor, λ_{GC}, estimates are given in **Supplementary Table 1a**.

Selection of SNPs for stage 2. We aimed in stage 2 to follow up SNPs which had evidence of association with PP or MAP and, for completeness, to evaluate the effects on PP and MAP of SNPs reported in recent large genome-wide association studies of SBP and DBP[1–3]. All SNPs with $P < 1 \times 10^{-5}$ for association with either PP or MAP (or both) were divided into independent regions based on linkage disequilibrium, and the most significant SNP was selected from each region. Within the *FIGN* region, different SNPs were associated with PP and with MAP, and both of these SNPs were followed up in stage 2. For SNPs with an *N* effective of <75% of the total *N*, a proxy was also included if it had $P < 1 \times 10^{-5}$ and $r^2 > 0.6$ with the top SNP (this occurred for one SNP). For all regions that had previously shown association with SBP or DBP[1–3], the sentinel SNP for PP and MAP and the previously reported SNP for SBP and DBP were followed up. In all, 99 SNPs were followed up in stage 2 (**Supplementary Note**) comprising: 44 SNPs from 22 loci with PP or MAP associations ($P < 1 \times 10^{-5}$) in stage 1 data and with previously reported SBP or DBP associations; 47 SNPs from 45 loci with PP or MAP associations ($P < 1 \times 10^{-5}$) in stage 1 data only and; 8 SNPs from 7 loci with previously reported SBP or DBP associations and no association ($P < 1 \times 10^{-5}$) with PP or MAP in the stage 1 data.

Stage 2. The characteristics of the stage 2 studies, including the genotyping and imputation approaches, are described in **Supplementary Table 1a**,**b**, and the details of the corrections for treatment are described in the **Supplementary Note**. For the 99 SNPs selected for follow-up, the stage 2 studies followed the analysis approach adopted in the stage 1 analyses. The meta-analysis was done using the inverse-variance weights method.

Pooled analysis of first- and second-stage samples. The meta-analysis from stages 1 and 2 was conducted using inverse-variance weighting, and genomic control was applied. A threshold of $P = 5 \times 10^{-8}$ was taken for genome-wide significance.

Calculation of risk scores. We calculated risk scores based on the most significantly associated SNP from all regions that were genome-wide significant after the meta-analysis of stages 1 and 2 for PP (10 SNPs) and MAP (22 SNPs) (**Supplementary Table 2f**). Each risk score was constructed using an approach described in the **Supplementary Note** and was tested for association with hypertension, coronary artery disease, stroke, hypertension, chronic kidney disease, heart failure and microalbuminuria and with the continuous traits of left ventricular mass, left ventricular wall thickness, pulse wave velocity, serum creatinine, eGFR and urinary albumin:creatinine ratio (**Supplementary Table 3**).

Additional analyses. Identification of potentially functional SNPs in linkage disequilibrium with the reported sentinel SNPs, expression quantitative trait loci analyses and expression analyses in human aortic samples were also carried out as discussed in the **Supplementary Note** and **Supplementary Figures 3** and **4**.

31. Li, Y. & Abecasis, G.R. Mach 1.0: Rapid haplotype reconstruction and missing genotype inference. *Am. J. Hum. Genet.* **S79**, 2290 (2006).
32. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39**, 906–913 (2007).
33. Servin, B. & Stephens, M. Imputation-based analysis of association studies: candidate regions and quantitative traits. *PLoS Genet.* **3**, e114 (2007).

doi:10.1038/ng.922